Detection of Stored-Grain Insect Infestation in Wheat Transported in Railroad Hopper-Cars

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ABSTRACT Levels of insect infestation, insect spatial distribution, and the relationship between the number of insect-damaged kernels (IDK) and the number of insects present in grain samples in three-hopper railcars transporting wheat from country elevators to a mill were studied. Six of eight sampled railcars were infested with more than two species of insects. The most abundant species collected were the lesser grain borer, *Rhyzopertha dominica* (F.), and rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), with the larval stage of the two species being the most prevalent (>90%). The spatial distributions of these two species within the grain mass were typically clumped in railcar compartments containing >0.4 insect/2.75-kg sample of wheat, and these foci of high-infestation levels varied in compartments within the railcars and among the sampled railcars. There were no significant correlations between IDK and insect density for any of the different stage-specific insect populations that were collected in the grain samples. Mean numbers of immatures and IDK differed among railcars and compartments within railcars, but not among grain depths. Number of insects in the first discharge sample was not correlated with mean numbers of insects in the entire compartment. This indicates that each compartment of a railcar should be sampled to determine level of insect infestation but that sampling at different depths within a compartment is less important.

KEY WORDS sampling, railcars, stored grain, insect-damaged kernels, spatial distribution

INSECT INFESTATION IS AN important quality factor of stored grain and represents a serious and continuing problem for the grain and milling industries. Acceptance of a specific grain lot by millers depends mainly on the numbers of live insects and insect-damaged kernels (IDK) detected before the grain is unloaded from a railcar. The most commonly used method for determining insect contamination and damage in railcars of grain is sampling with a grain trier, sieving insects from the sample, and visual inspection of a portion of the sample for insect-damaged kernels. Grain buyers especially need to make rapid decisions about the quality of individual lots of grain to accept or reject the product. However, there are indications from the buyers that the method used to detect infestations may not be accurate.

A variety of grain sampling devices are available commercially in the United States for detection of insects in stored grain. Compartmented brass or aluminum grain triers, probes, and spears have been widely used to take samples from bulk grain in on-farm storage bins, trucks, railcars, barges, and ship holds (Meagher et al. 1986, Hagstrum 1994). These devices collect grain samples of 0.5–1.0 kg, generally in the upper areas of the grain mass (Meagher et al. 1986). Mechanical samplers, which include pneumatic grain samplers, are available for taking samples from deep within the grain mass (Hagstrum 1994). The sampled grain is sieved, and the insects passing through the screen are counted. These methods are widely used by farmers and grain buyers to determine the extent of infestation (Wright and Mills 1983) because they give a quick indication of the number and species of insects that are present in the grain sample (Wilkin and Fleurat-Lessard 1990, Reed et al. 1991, Hagstrum 1994). However, these methods also have been reported to be inaccurate when detecting population densities below four insects per kilogram (Wright and Mills 1983, White and Loschiavo 1986, Wilkin and Fleurat-Lessard 1990), and they do not indicate the presence of insects feeding inside kernels. These internally feeding insects are responsible for insect fragments in products produced from the grain because internally feeding insects cannot be easily cleaned from the grain during processing. If the grain will be stored before use, then these internally feeding insects may quickly develop and reproduce, resulting in loss of quality of the stored grain.

To detect insect contamination in railcars, millers normally take one subsample ($\approx$0.5 kg) with a hand trier from each of the three compartments in a railcar and then combine the three subsamples from each car. The composite grain sample is sieved, and the numbers of live insects are recorded. Loads are often rejected or accepted based on this sampling technique, but the probability of detecting insect infestation from one grain sample is very low. For example, if only one grain sample per 1000 bushels is taken, the probability of detecting a mean density of two insects per kilogram is 76% (Hagstrum and Flinn 1992). Assuming that a railcar holds between 3,000 and 3,500 bu of wheat, this probability is reduced to $\approx$25%. Sometimes, millers also take another grain sample at the hopper bottom of each railcar compartment, as the grain is unloaded. These methods used to sample railcars will only detect insects external to the grain and do not detect the internally feeding stages. Methods for detecting these internally feeding insects, such as x-ray (Karunakaran et al. 2003), enzyme-linked immunosorbent assay (Brader et al. 2002), or near-infrared spectroscopy (Dowell et al. 1998) can be either inaccurate, time-consuming, or they can only detect older immature insects.

Insect-damaged kernels provide a direct measure of insect damage to kernels. For IDK determination, a 100-g subsample is taken from the composite sample from a railcar and is visually inspected for insect-damaged kernels. Because such a small amount of grain is inspected, U.S. grain mills will normally not accept wheat shipments with levels >5 IDK/100 g because they believe that higher IDK levels indicate that insect fragment levels in the flour milled from the grain will be too high (Flinn and Hagstrum 2001). However, there are no published data to support this assumption.

In the United States, the Federal Grain Inspection Service (FGIS) has established the term "infested" for lots of wheat containing two or more live insects injurious to grain per 1000 g (FGIS 2003). The Food and Drug Administration (FDA) has established that the presence of live insects in stored bulk grains is considered to be an adulteration. The FDA has set the defect action level (DAL) as the regulatory standard for quality control. For insect contamination, the DAL is 32 or more insect-damaged kernels per 100 g of wheat (FDA 2003). However, U.S. millers routinely reject wheat that has much lower levels of IDK than the DAL to ensure compliance with the federal requirement and to deliver high-quality flour to their customers.

The objective of this study was to evaluate the utility of current sampling methods for making a decision to accept or reject a load of grain. To meet this objective, we compared the insect density estimates and IDK measurements of discharge samples taken from the hopper bottom with those generated by doing a complete assessment of insect levels and IDK from samples collected using a pneumatic grain sampler. The specific objectives were to 1) determine whether IDK counts are indicative of insect infestation levels, 2) determine the age structure of the insect population in the infested railcars, and 3) determine the spatial distribution of insects and IDK in the grain mass.

Materials and Methods

Selection of Railcars. This study was performed in collaboration with a flour mill in Kansas City, MO. Grain was sampled from inbound three-hopper railcars carrying $\approx$100 tons of hard red winter wheat, *Triticum aestivum* L. Each railcar had three independent compartments, with dimensions of 5.5 m (18 feet) in length by 4.0 m (13 feet) in height by 3.2 m (10 feet) in width, in which the grain was held. Eight railcars were sampled, two railcars on each of four dates in 2002, at the plant before unloading on 24 September (railcars 1 and 2), 9 October (railcars 3 and 4), 30 October (railcars 5 and 6), and 3 December (railcars 7 and 8). Railcars with a range of IDK values (determined by the seller at the origin point) were provided by the miller: four railcars with 0–1 IDK/ 100 g of wheat (cars 1–4), two railcars with 2–3 IDK/ 100 g (cars 5 and 6), and two railcars with six or more IDK/100 g (cars 7 and 8). The two cars sampled on a given date were from the same origin. However, we did not know the origin of the grain or the distance that the railcars traveled.

Grain Sampling. Grain from each railcar was probed at six evenly spaced sampling points in each of the three compartments by using a pneumatic (vacuum) grain sampler (Cargill Probe-A-Vac, Minneapolis, MN) fitted with 91.5-cm (3-foot) probe sections: two sampling points in the front of the compartment (91.5 cm [3 feet] from the edge), two near the center (2.75 m [9 feet] from the front edge), and two at the back of the compartment (4.6 m [15 feet] from the front edge) on either side of the centerline. The samples were taken as far from the centerline ($\approx$46 cm [1.5 feet] on either side from the centerline) as the hatch opening allowed and angled slightly toward the closest side.

Grain sample portions of $\approx$2.75 kg were taken in each sampling point at three different grain depths: at 0–0.915 m (0–3 feet), 0.915–1.85 m (3–6 feet), and 1.85–2.75 m (6–9 feet) beneath the grain surface. Eighteen vacuum probe samples were taken in each compartment, except in cars 2, 4, and 7, in which compartment 3 was not completely full of grain so that only 12 grain samples were taken from each of these compartments (no samples from the surface). In addition, three samples were taken as the grain flowed out of the hopper bottom of each compartment. These discharge samples were taken at the beginning, at mid-way, and near the end of the unloading process for each compartment. Samples were placed in labeled plastic 3.8-liter (1-gallon) jars, transported to the laboratory, and stored in a walk-in rearing chamber held at 27°C, 65 $\pm$ 2% RH, and a photoperiod of 12:12 (L:D) h.

The temperature of the grain was measured before wheat samples were taken from each compartment

using a Digi-Sense thermistor thermometer (Cole-Parmer Instrument Co., Chicago, IL). Grain temperature was measured at 1-m depth in the center of each compartment by inserting a metal rod, with a thermistor attached at the end, into the grain.

Grain Sample Analysis. The day after collection, each sample was processed with an Insectomat, a motorized inclined sieve (89 by 43 cm, 1.6-mm aperture) (Samplex Ltd., Willow Park, United Kingdom), by passing the sample once over the sieve to remove all of the live and dead insects present outside of the grain kernels. Adult insects were then separated from the fine material that was removed during screening, and the fine material and immature insects were returned to the grain sample in the plastic jars. One 100-g subsample was taken from each sample and sent to the miller for IDK analysis by a private contractor. The samples were returned to the rearing chamber to determine the presence of insects inside kernels and the age structure of the population. The grain samples were sieved again after 1, 4, and 7 wk to remove any insects that emerged from inside the grain kernels. All of these insects that emerged from kernels were classified as "immatures" in the statistical analysis. Adult insects were identified, and the number of live and dead insects was recorded by species.

Statistical Analyses. Variation in population densities of live adults, dead adults, immatures, total numbers of insects, and IDK numbers among railcars, compartments within railcars, and within different depths in the grain mass were analyzed separately for pneumatic trier samples and discharge samples by using analysis of variance (ANOVA). PROC GLM (SAS Institute 1998) was used to prepare fixed effects models to test for random effects (railcars, compartments, and interactions). PROC MIXED (SAS Institute 1998) was used to test for fixed effects (depths and sampling method). ANOVA (PROC GLM, SAS Institute 1998) also was used to determine which insect species were found most frequently in the grain samples. We found residues of aluminum phosphide tablets on the grain surface of railcars 5 and 6 (30 October), indicating that the grain was fumigated in transit. No live or dead insects were found in grain samples from those railcars, so data from those railcars were excluded from the analyses. The relationships between mean number of live adults, dead adults, immatures, total insect population, and IDK in vacuum samples versus number of live adults, dead adults, immatures, third instar and older insects inside the kernels, total insect populations, and IDK in the first discharge sample were determined by linear correlation (PROC CORR, SAS Institute 1998) and by stepwise regression (PROC REG, SAS Institute 1998). We used the sequential Bonferroni technique (Rice 1989) to control the error rate for the 30 correlations at the 0.05 level. We also compared the relationships between IDK and number of insects in the grain mass and between adult and immature stages for each sampling method by linear correlation, and, again, we used the sequential Bonferroni technique to control the error rate at the 0.05 level.

To assess dispersion of sample captures, the standardized Morisita index (Krebs 1999) of dispersion (I_p) was calculated for grain sample data of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), and the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae). This index is independent of population density and sample size and, in its standardized form, ranges from -1.0 to $+1.0$ (95% confidence intervals of -0.5 and $+0.5$). With this measure of dispersion, $I_p = 0$ indicates a random pattern, $I_p > 0$ indicates a clumped pattern, and $I_p < 0$ indicates a uniform pattern.

Results

Grain Temperature

The temperature of the grain in railcars sampled in September averaged 24.8°C but decreased to 21.3°C in railcars sampled at the end of October and to 15.7°C in railcars sampled in the beginning of December (Fig. 1).
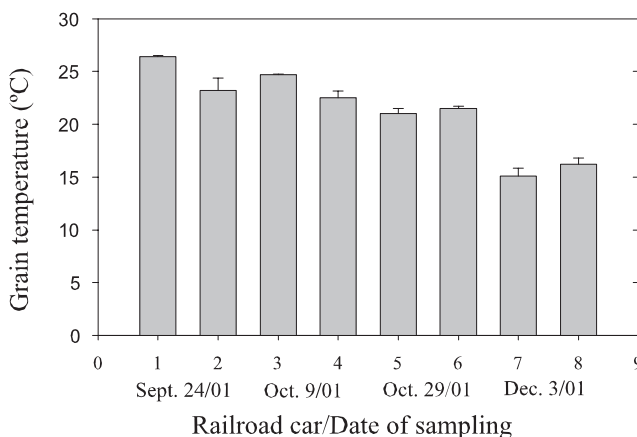

Fig. 1. Mean ± SEM grain temperatures (°C) recorded in wheat in railcars sampled on four dates from September to December 2001.

Table 1.   Insect population densities and insect-damaged kernels (IDK) in wheat transported in railroad cars from country elevators to the receiving area of a milling plant

Cars	No. insects per 2.75-kg sample of wheat (mean ± SEM)							
	Live adults		Dead adults		Immatures		IDK	
	Vacuum	Discharge	Vacuum	Discharge	Vacuum	Discharge	Vacuum	Discharge
1	0.30 ± 0.11	0.78 ± 0.55	0.35 ± 0.12	0.44 ± 0.18	13.30 ± 1.72	20.67 ± 5.40	0.40 ± 0.07	0.67 ± 0.17
2	0.00 ± 0.00	0.11 ± 0.11	0.13 ± 0.02	0.33 ± 0.17	0.40 ± 0.15	1.22 ± 0.64	0.40 ± 0.08	0.22 ± 0.15
3	0.00 ± 0.00	0.11 ± 0.11	0.80 ± 0.13	4.67 ± 2.06	0.02 ± 0.01	0.44 ± 0.24	0.17 ± 0.06	0.22 ± 0.15
4	0.04 ± 0.02	0.11 ± 0.11	0.50 ± 0.11	0.22 ± 0.13	0.85 ± 0.19	7.90 ± 1.22	0.35 ± 0.08	0.33 ± 0.24
7	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.01	0.00 ± 0.00	0.06 ± 0.04	0.22 ± 0.22	6.98 ± 0.50	5.67 ± 0.24
8	0.02 ± 0.01	0.00 ± 0.00	0.06 ± 0.01	0.11 ± 0.11	1.11 ± 0.19	4.33 ± 3.40	6.19 ± 0.43	3.00 ± 0.24

Insect Density and Insect-Damaged Kernels

Vacuum Samples. The mean number of live adults in the initial vacuum samples did not differ among railcars ($F = 4.3$; df = 5, 3.3; $P = 0.12$), among compartments within railcars ($F = 0.5$; df = 10, 18.2; $P = 0.85$), or among grain depths ($F = 0.44$; df = 2, 4; $P = 0.67$). The interactions railcar × depth ($F = 1.5$; df = 10, 18.1; $P = 0.23$), compartment × depth ($F = 1.0$; df = 4, 18.6; $P = 0.44$), and compartment × depth × railcar ($F = 1.5$; df = 18, 254; $P = 0.08$) were not significant. Live adults were present in vacuum samples from only three railcars, and the mean numbers per sample varied from 0.0 to 0.3 (Table 1).

The mean number of dead adults in the initial vacuum samples differed among railcars ($F = 5.3$; df = 5, 12.9; $P < 0.01$) and the interaction railcar × depth ($F = 4.3$; df = 10, 18.3; $P < 0.01$) was significant; but, the mean number of dead adults did not differ among compartments within railcars ($F = 2.3$; df = 10, 18.8; $P = 0.06$) or among grain depths ($F = 0.3$; df = 2, 4; $P = 0.78$), and the interactions compartment × depth ($F = 0.5$; df = 4, 20.5; $P = 0.71$) and compartment × depth × railcar ($F = 0.4$; df = 18, 254; $P = 0.99$) were not significant. Dead adults were present in the six railcars, and the mean numbers per sample varied from 0.04 to 0.8 (Table 1). We looked more closely at the railcar × depth interaction, but there was no obvious pattern to the numbers of dead adults at each depth in individual railcars.

The mean number of immatures present in the initial vacuum samples differed among railcars ($F = 4.8$; df = 5, 17.2; $P < 0.01$) and among compartments within railcars ($F = 14.4$; df = 10, 18.4; $P < 0.01$) but not among grain depths ($F = 0.5$; df = 2, 4; $P = 0.65$). The interaction railcar × depth was significant ($F = 8.6$; df = 10, 18.1; $P < 0.01$), but the interactions compartment × depth ($F = 1.3$; df = 4, 19.1; $P = 0.30$) and compartment × depth × railcar ($F = 0.8$; df = 18, 254; $P = 0.71$) were not significant. Immature insects were present in the six railcars. Mean number of immatures per 2.75-kg sample varied from 0.02 to 13.3 (Table 1). The number of immatures varied significantly among the three compartments in the same railcar. For example, in railcar 1, the mean number of immatures per sample varied from 5.5 ± 1.5 insects in compartment 1 to 18.9 ± 3.1 and 17.1 ± 2.8 insects in compartments 2 and 3, respectively. In railcar 8, the mean numbers of immatures per sample varied from 2.1 ± 0.4 insects

in compartment 1 to 0.8 ± 0.2 and 0.5 ± 0.2 insects in compartments 2 and 3, respectively. We looked more closely at the railcar × depth interaction, but there was no obvious pattern to the numbers of immatures at each depth in individual railcars.

The mean number of insect-damaged kernels in the initial vacuum samples differed among railcars ($F = 20.3$; df = 5, 11.1; $P < 0.01$) and among compartments within railcars ($F = 7.6$; df = 10, 18.3; $P < 0.01$), but not among grain depths ($F = 1.5$; df = 2, 4; $P = 0.34$). The interactions railcar × depth ($F = 1.6$; df = 10, 18.1; $P = 0.19$), compartment × depth ($F = 0.1$; df = 4, 18.9, $P = 0.97$), and compartment × depth × railcar ($F = 1.0$; df = 18, 254; $P = 0.48$) were not significant. The mean IDK values varied from 0.17 to 7.0 per 100-g sample (Table 1). The number of insect-damaged kernels varied among compartments in the same railcar. The largest differences among compartments were in railcar 8, where the mean IDK per sample varied from 4.2 ± 0.3 and 4.9 ± 0.4 in compartments 1 and 2, respectively, to 9.4 ± 0.8 IDK in compartment 3.

Discharge Samples. The mean number of live adults in the discharge samples did not differ among railcars ($F = 1.3$; df = 5, 12; $P = 0.35$) or compartments within railcars ($F = 1.4$; df = 12, 36; $P = 0.23$). Live adults were present in discharge samples from the first four railcars. The mean numbers of live adults in the discharge samples varied from 0.0 to 0.78 per 2.75-kg sample (Table 1).

The mean number of dead adults in the discharge samples did not differ among railcars ($F = 1.3$; df = 5, 12; $P = 0.32$), but it did differ among compartments within railcars ($F = 18.7$; df = 12, 36; $P < 0.01$). Dead adults were found in five of the six railcars. The mean number per sample varied from 0.0 to 4.7 (Table 1). The largest differences among compartments were in railcar 3, where the mean number of dead adults per sample varied from 12.3 ± 2.4 in compartment 1 to 0.0 ± 0.0 and 2.0 ± 1.0 in compartments 2 and 3, respectively.

The mean number of immatures in the discharge samples differed among railcars ($F = 3.3$; df = 5, 12; $P = 0.04$) and among compartments within railcars ($F = 5.7$; df = 12, 36; $P < 0.01$). The mean number of immatures varied from 0.2 to 20.7 insects per 2.75-kg sample (Table 1). There were large differences in the number of immature insects per sample among some compartments in the same railcar. In railcar 1, the

mean number of immatures per sample in compartment 1 was 2.7 ± 0.3 compared with 35.3 ± 2.2 and 24.0 ± 8.3 immatures in compartments 2 and 3, respectively. There were also large differences among compartments in railcar 8, where the mean number of immatures per sample varied from 0.00 to 0.3 ± 0.1 insects in compartments 1 and 2, respectively, and 12.7 ± 9.1 insects in compartment 3.

The mean number of insect-damaged kernels in the discharge samples differed among railcars ($F = 104.5$; $df = 5, 12$; $P < 0.01$) but not among compartments within railcars ($F = 1.3$; $df = 12, 36$; $P = 0.27$). The mean number of IDK per 100-g sample varied from 0.22 to 5.7 (Table 1).

There were no significant differences in the number of live insects ($F = 1.3$; $df = 2, 10$; $P = 0.33$), dead insects ($F = 1.2$; $df = 2, 10$; $P = 0.34$), immature insects ($F = 0.6$; $df = 2, 10$; $P = 0.56$), or IDK ($F = 0.8$; $df = 2, 10$; $P = 0.48$) among the three discharge samples from each compartment. For example, only five of the 54 discharge samples contained live adults, and two of those were first discharge samples, two were second samples, and one was a third discharge sample.

Both sampling methods (vacuum and discharge samples) were able to detect the presence of insects in grain transported in railcars. No significant differences were found between the two methods for the number of live adults ($F = 4.4$; $df = 1, 5$; $P = 0.09$), dead adults ($F = 1.0$; $df = 1, 5$; $P = 0.36$), or IDK ($F = 1.7$; $df = 1, 5$; $P = 0.25$) present in samples at collection. The number of immatures that emerged from vacuum and discharge samples after 7 wk also did not differ ($F = 6.1$; $df = 1, 5$; $P = 0.06$).

Stepwise regression showed that only one factor at a time was significant for predicting the relationships between mean number of live adults, dead adults, immatures, total insect population, and IDK in vacuum samples versus number of live adults, dead adults, immatures, third instar and older insects inside the kernels, total insect populations, and IDK in the first discharge sample. Thus, we used correlation analyses to explore these relationships. From the 30 correlation analyses between vacuum samples and the first discharge sample, there were 10 correlations that were significant. However, only the relationship between
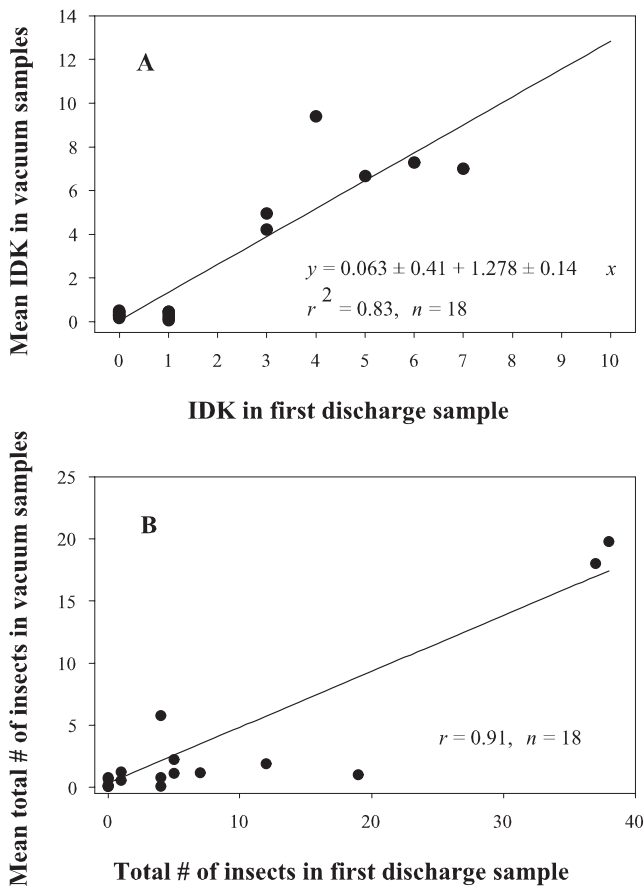

$$y = 0.063 \pm 0.41 + 1.278 \pm 0.14 \quad x$$
$$r^2 = 0.83, \quad n = 18$$

$$r = 0.91, \quad n = 18$$

Fig. 2. Relationship between IDK or insects in first 2.75-kg discharge sample and mean IDK or insects in 2.75-kg vacuum samples. (A) IDK in first discharge sample versus mean IDK in vacuum samples. (B) Total numbers of live insects in first discharge sample versus mean total numbers of live insects in vacuum samples. Parameter values for the equation in A are reported as slope or intercept ± SE.
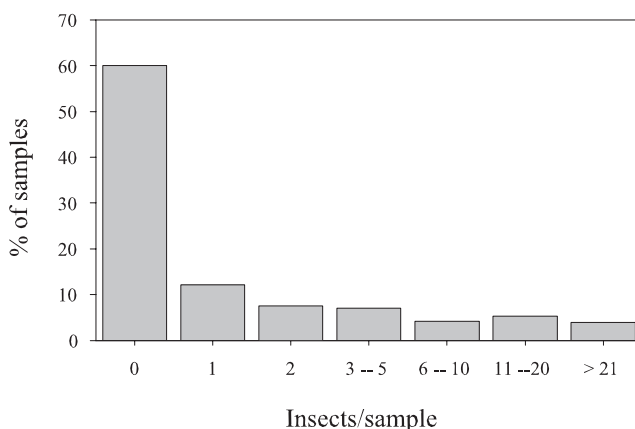
Fig. 3. Frequency distribution of live (immatures and adults) insects in 2.75-kg wheat samples (vacuum and discharge samples) collected in railcars.

IDK in vacuum samples versus IDK in the first discharge sample seemed useful (Fig. 2A), indicating that it is possible to predict IDK in the whole railcar compartment by measuring IDK in the first discharge sample. Other significant correlations were between number of immatures, number of third instar and older insects inside kernels, or total number of insects in the first discharge sample and mean number of live adults, immatures, or total number of insects in the vacuum samples. However, although statistically significant, the significance always was based on two discharge samples from compartments 2 and 3 of car 1 that had high densities of insects, as illustrated in Fig. 2B. When these two samples were excluded from the analyses, the r values (which originally ranged from 0.84 to 0.96 for these nine correlations) fell to 0.05–0.37.

Occurrence of Insect Species

The six railcars were infested with more than two species when considering only live immature and adult stored-grain insects recovered from samples. About 40% of the samples collected from railcars were infested with live insects (113 of 306 vacuum and 30 of 54 discharge samples were infested with live immature

or adult insects), but ≈30% of those infested samples contained only one insect (Fig. 3). The most common species found in the grain samples was *R. dominica*, which was found in the six railcars and represented 84.5% of the total live insects recovered. *R. dominica* was the predominant species in railcars 1, 2, 3, and 8 (Table 2). The second most common species was *C. ferrugineus*, which also was found in the six railcars and represented 14.2% of the total insect population found in the railcars. This was the predominant species in railcars 4 and 7 (Table 2). Other stored-grain pests found were beetles (Coleoptera): rice weevil, *Sitophilus oryzae* (L.) (Curculionidae); red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae); foreign grain beetle, *Ahasverus advena* (Waltl) (Silvanidae); hairy fungus beetle, *Typhaea stercorea* (L.) (Mycetophagidae); sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Silvanidae); and dermestid larvae (Dermestidae). These species represented only ≈1.3% of the total population of live insects in the samples.

Dead adult insects were present in vacuum and discharge samples at sampling time. Most of these were *T. castaneum* (57.5% of the total), *R. dominica* (25.8%), or *C. ferrugineus* (12.5%).

Based on the duration of development of the different stages of *R. dominica* at 26°C and 70% RH (Birch 1945), *R. dominica* that emerged at 1 wk after sampling were considered to be pupae and preemerged adults at the time of sampling; adults that emerged at 4 wk after sampling were considered to be second, third, and fourth instars at sampling; and insects that emerged at 7 wk after sampling were considered to be eggs and first instars at sampling. Thus, the age structure of this species at sampling was eggs and first instars, 50.7%; second, third, and fourth instars, 42.2%; pupae and preemerged adults, 4.8%; and adults, 2.3%. About 40% of the samples were infested only with eggs and first instars at sampling, whereas ≈10% of the samples were infested with all of the stages of *R. dominica* at sampling (Fig. 4).

Table 2. Occurrence of live stored-grain insect species in wheat transported in railroad cars from country elevators to the receiving area of a milling plant

Cars	No. insects per 2.75-kg sample of wheat (mean ± SEM)[a]		
	R. dominica	*C. ferrugineus*	Others[b]
1	13.76 ± 1.67	0.36 ± 0.07	0.07 ± 0.05
2	0.46 ± 0.16	0.02 ± 0.01	0.02 ± 0.01
3	0.07 ± 0.03	0.02 ± 0.01	0.03 ± 0.02
4	0.46 ± 0.11	1.35 ± 0.29	0.02 ± 0.01
7	0.00 ± 0.00	0.07 ± 0.04	0.02 ± 0.01
8	0.97 ± 0.50	0.57 ± 0.10	0.03 ± 0.02

[a] Represents live immatures and adults in vacuum and discharge samples.
[b] Includes *S. oryzae, T. castaneum, T. stercorea, O. surinamensis, A. advena*, and dermestids.
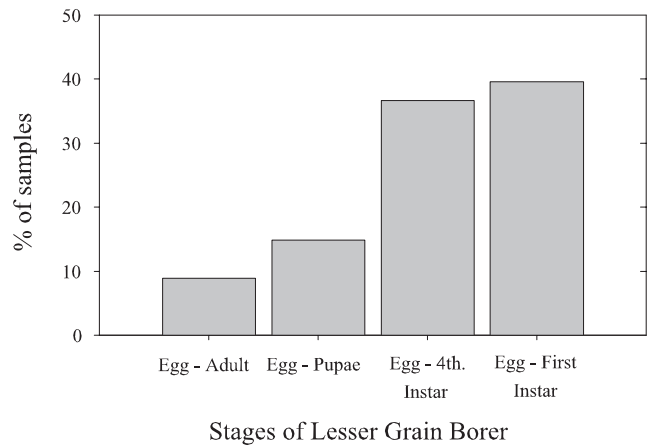
Fig. 4. Percentage of 2.75-kg wheat samples (vacuum and discharge samples) infested simultaneously with different stages of *R. dominica* at sampling time.

For *C. ferrugineus* (Rilett 1949), insects that emerged at 1 wk after sampling were considered to be pupae at the time of sampling; adults that emerged at 4 wk after sampling were considered to be first, second, third, and fourth instars at sampling; and insects that emerged at 7 wk after sampling were considered to be eggs at sampling. Thus, the age structure of this species at sampling was 19.7% eggs, 70.7% larvae, 4.8% pupae, and 4.8% adults.

Distribution of Insects in Grain Mass

The dispersion of *R. dominica* among vacuum samples was clumped (Morisita indexes >0.5) for compartments with the highest insect population densities (mean population density >0.4 insects per sample), whereas in compartments with the lowest population

densities (mean population density <0.4 insects per sample), the dispersion of individuals among grain samples was random (Morisita indexes = 0) (Table 3). The location of high-density patches of *R. dominica* within a compartment varied among compartments even within the same railcar. For example, in railcar 1, 73% of individuals were found in the upper rear of compartment 1, whereas 74% of individuals were found in the upper front and middle rear in compartment 2, and 56% were close to the middle bottom in compartment 3. The dispersion of *C. ferrugineus* among grain samples also was clumped in the compartments with the highest population densities and random in compartments with the lowest population densities (Table 3). *C. ferrugineus* recovery tended to be highest in samples in the center portion of the compartments.

Table 3. Standardized Morisita index of dispersion (I_p) for insects in 2.75-kg samples of wheat (vacuum samples) from railroad cars

Railroad car	Compartment	R. dominica	C. ferrugineus
1	1	0.530[a]	0.0
1	2	0.508[a]	−0.160
1	3	0.506[a]	0.0
2	1	0.517[a]	0.0
2	2	0.0	—
2	3	—	0.0
3	1	0.130	—
3	3	—	0.0
4	1	0.498	0.524[a]
4	2	0.490	0.521[a]
4	3	0.507[a]	0.548[a]
7	2	—	0.458
7	3	—	0.0
8	1	0.635[a]	0.479
8	2	0.0	0.466
8	3	0.0	0.499

The index of dispersion ranges from −1.0 to 1.0, where random patterns have an I_p = 0, clumped patterns have an I_p > 0, and uniform patterns have an I_p < 0. The 95% confidence limits for a random pattern of distribution are at −0.5 and +0.5 of the I_p value.

[a] Mean population density >0.4.

Relationship between IDK and Presence of Insects in Grain

There was no relationship, based on the sequential Bonferroni method (Rice 1989), between IDK and the presence of adult internal feeders in both vacuum ($r = -0.07$, $n = 306$) and discharge ($r = -0.18$, $n = 54$) samples; between IDK and number of adult external feeders ($r = -0.21$, $n = 306$; $r = -0.24$, $n = 54$); between IDK and number of larval internal and external feeders ($r = -0.17$, $n = 306$; $r = -0.19$, $n = 54$); or between IDK and the total insect population ($r = -0.19$, $n = 306$; $r = -0.25$, $n = 54$). Figure 5 shows the relationships between IDK and insect population in vacuum samples. Samples with no IDK contained 0–48 insects per sample, and samples with no insects contained 0–18 IDK.

Discussion

Wheat temperatures were generally favorable for insect survival, development, and reproduction in the first six railcars sampled (Howe 1965, Hagstrum 1987).
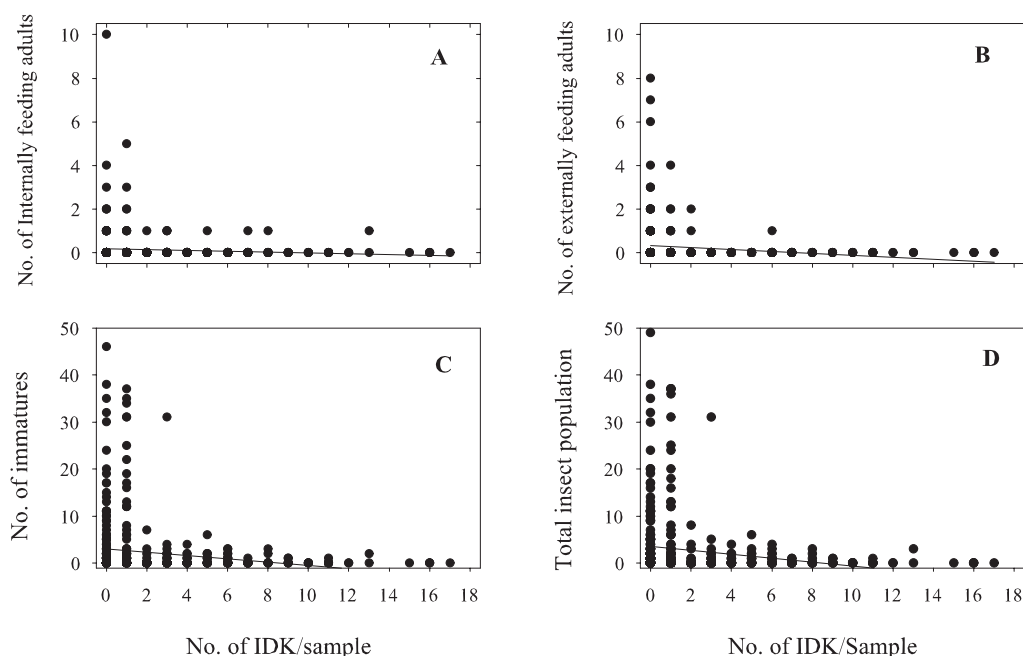
Fig. 5. Relationship between numbers of IDK and stored-grain insects in 2.75-kg vacuum samples collected from railcars. (**A**) IDK versus internally feeding adults. (**B**) IDK versus externally feeding adults (**C**) IDK versus immatures. (**D**) IDK versus total insect population.

Temperature of the wheat in the last two railcars sampled dropped to ≈15°C, but still this temperature was favorable for survival of the two more abundant species found in the grain samples, *C. ferrugineus* (Evans 1983) and *R. dominica* (Hagstrum and Flinn 1994).

About 53% of the grain samples collected from the railcars with both sampling methods (vacuum or discharge) were infested with live or dead insects. Several researchers have reported the same tendency. Cogburn (1973) found that 81% of grain samples from railcars transporting rice were infested with one or more species of stored-product insects [*T. castaneum*, dermestids, *C. ferrugineus, Ephestia cautella* (Walker), and *R. dominica*]. Smith and Loschiavo (1978) reported that 26% of 340 grain samples collected in western Canada from four railcars transporting wheat were infested with *C. ferrugineus* and *T. castaneum.*

Eight species of stored-product insect pests were collected from our grain samples. The two most abundant species were *R. dominica* (develops internally) and *C. ferrugineus* (develops externally). These two species are the most common species in stored wheat in the United States (Hagstrum 1987, Dowdy and McGaughey 1994). *R. dominica* was present in 54% of the compartments sampled, followed by *C. ferrugineus* (50%), *S. oryzae* (12.5%), dermestids (12.5%), *O. surinamensis* (8.3%), and *T. castaneum, T. stercorea,* and *A. advena* (4.2%). The insect density ranged from 1 to 21 live adults and from 4 to 761 live immatures in ≈175 kg of wheat sampled per railcar, or from 0.006 to 0.120 live adults per kilogram of wheat and from 0.023 to 4.35 live immatures per kilogram of wheat.

Adult *R. dominica* were found in two of the eight sampled railcars and represented only 2% of the total lesser grain borer population; whereas, immature *R. dominica* were present in five of the sampled railcars and represented 98% of the total *R. dominica* population. Adult and immature *C. ferrugineus* were found in four railcars and represented 5 and 95% of the *C. ferrugineus* beetle population, respectively.

Sometimes, grain handlers take one discharge sample from the hopper bottom at the beginning of the unloading process to determine whether the grain is infested with insects. They take this sample because they think that insects tend to concentrate at the bottom of the compartment as a result of vibration during transit. This assumption was not supported by the results of our study: the two most abundant stored-product insects found in the grain samples varied considerably in distribution within the compartments. Increasing the number of discharge samples from the hopper bottom of each compartment from one to three, improved the possibility of detecting insect infestation. In our study, we took one discharge sample immediately upon opening the gate, one at the middle, and the last at close to the end of unloading the grain. With this method, detection of the presence of insects did not differ from vacuum sampling. Collecting grain samples as the grain is unloaded is less labor- and time-intensive; however, this method has the disadvantage that millers cannot make the decision to accept or reject lots of grain before unloading.

We used correlation analysis to investigate the relationship between live total internal feeding adult insect density in the grain samples and IDK, total

external feeding adults density and IDK, total larval density and IDK, and total (live adults + larvae) insect density and IDK in both vacuum and discharge samples. None of these correlations were significant. The number of IDK was higher in railcars 7 and 8. In contrast, live adult population density was higher in railcars 1, 2, 3, and 4. The same was true for dead insects. A possible explanation for this lack of correlation may be due to the use of fumigation before and during shipment and separation of external adults, damaged kernels, and internally infested kernels during the grain movement associated with loading railcars. Therefore, number of insect-damaged kernels was not a reliable indicator of the degree of insect infestation. This confirmed the results of Russell (1988) who showed, using the x-ray method, that wheat samples with <32 IDK per 100 g (visual examination) have enough internal insects to produce flour with insect fragment counts more than the FDA defect action level (75 fragments/50 g of flour).

In our study, the mean number of immatures and IDK differed among railcars and compartments within railcars, but not among grain depths. This indicates that each compartment of a railcar should be sampled to determine level of insect infestation, but that sampling at different depths within a compartment is less important.

Visual examination of grain samples provides information about the population of adult and immature external feeders and the number of adult internal feeders present in the grain sample, but it does not provide information about the number of immature internal feeders infesting the grain (Hagstrum 1994). Our data showed that >90% of the *R. dominica* population infesting the grain was in immature stages. Therefore, if we had just sieved samples for external insects, we would have missed >90% of the total population of *R. dominica* infesting the grain at sampling. To detect these insects inside the kernels, it was necessary to incubate the grain samples for a 7-wk period. This is not a good alternative for grain handlers who have to make rapid decisions about the quality of individual lots or loads of grain. A new method based on near-infrared spectroscopy has been developed for detection of immature insects inside wheat kernels (Dowell et al. 1998). However, this method, and other rapid detection methods, can detect only third instar and older insects inside kernels. These rapid detection methods would have detected infestations in 60% of our infested samples, but it would not have detected infestations in the 40% of infested samples that contained only eggs or first instars (Table 4). When samples contained only one *R. dominica*, 87% of those samples were infested only with eggs or first instars; thus, only 13% of those samples would be correctly classified by rapid detection techniques. However, detection efficiency increases greatly in more heavily infested samples. Percentage of correct classification increased to 54% when there were two *R. dominica* per sample, to 71% when there were three to five *R. dominica* per sample, and to >90% when there were six or more *R. dominica* per sample.

Table 4. Percentage of 2.75-kg samples of wheat (vacuum and discharge) that would have been correctly classified as infested by rapid detection techniques that detect third instar and older internal feeders inside the kernels

No. insects/ sample	No. samples infested with				% correctly classified
	Eggs to 1st instars	Eggs to 4th instars	Eggs to pupae	Eggs to adults	
1	27	3	1	0	12.9
2	6	4	2	1	53.8
3–5	5	12	0	1	70.6
6–10	0	7	1	0	100
11–20	1	7	7	2	94.1
>21	1	4	4	5	92.9
All infested samples	40	37	15	9	60.7

Our data showed that there was no correlation between IDK and the insect infestation level present in the grain, especially immature stages of internally feeding insects. This finding has implications for the resulting number of insect fragments in flour processed from the grain. Immatures that develop inside the kernels are the most important source of insect fragments because they cannot be removed from the grain by normal cleaning procedures (Sachdeva 1978).

Our data also showed that numbers of insects in the first discharge sample were not correlated with number of insects in the entire compartment. There were indications in the correlation analyses that one might predict number of immatures or live adults in the entire compartment based on number of third instar and older insects inside kernels, immatures, or total number of insects in the first discharge sample, but these significant correlations were strongly influenced by high insect counts from only two compartments. When those two data points were excluded, the correlations were no longer significant. Most of the compartments had very low insect counts in the first discharge sample, and our results show that these low counts in the discharge samples are not correlated with insect counts in the rest of the compartment.

Currently, no economical and rapid methods exist for detecting all stages of insects that develop internally in stored grain. Grain millers and managers could benefit greatly if a rapid sampling technology were developed.

Acknowledgments

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